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ORIGINAL ARTICLE

Repetitive hyperbaric oxygen therapy provides better effects on brain inflammation and oxidative damage in rats with focal cerebral ischemia

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Background/Purpose: Repetitive hyperbaric oxygen (HBO_2) therapy may cause excessive generation of reactive oxygen species. This study assessed whether repetitive or 2—4-day trials of HBO_2 therapy (2 treatments daily for 2—4 consecutive days) provides better effects in reducing brain inflammation and oxidative stress caused by middle cerebral artery occlusion (MCAO) in rats than did a 1-day trial of HBO_2 therapy (2 treatments for 1 day).
Methods: Rats were randomly divided into four groups: sham; MCAO without HBO_2 treatment; MCAO treated with 1-day trial of HBO_2; and MCAO treated with 2—4-day trials of HBO_2. One treatment of HBO_2 (100% O_2 at 253 kPa) lasted for 1 hour in a hyperbaric chamber.
Results: Therapy with the 2—4-day trials of HBO_2 significantly and dose-dependently attenuated the MCAO-induced cerebral infarction and neurological deficits more than the 1-day trial of HBO_2 therapy. The beneficial effects of repetitive HBO_2 therapy were associated with: (1) reduced inflammatory status in ischemic brain tissues (evidenced by decreased levels of tumor necrosis factor-α, interleukin-1β, and myeloperoxidase activity); (2) decreased oxidative damage in ischemic brain tissues (evidenced by decreased levels of reactive oxygen and nitrogen species, lipid peroxidation, and enzymatic pro-oxidants, but increased levels of enzymatic antioxidant defenses); and (3) increased production of an anti-inflammatory cytokine, interleukin-10.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Conclusion: The results provide the apparently contradictory finding that heightened oxygen tension reduced oxidative stress (and inflammation), which was reflected by increased antioxidant and decreased oxidant contents under focal cerebral ischemia.

Introduction

Hyperbaric oxygen (HBO_2) therapy at a single dose is associated with decreased brain neurtrophil infiltration[1] and myeloperoxidase activity[2] in middle cerebral artery occlusion (MCAO). In experimental MCAO, one dose of HBO_2 therapy is also associated with downregulation of cyclooxygenase-2 mRNA and protein levels (an inducible enzyme responsible for elaboration of inflammatory prostanoids, prostaglandins, prastacyclins, and thromboxane),[3] suggesting that single HBO_2 therapy may improve outcomes of MCAO by reducing brain inflammation. In addition, inhibition of reactive oxygen species (ROS) production by an antioxidant is found to be beneficial in treating MCAO rats.[4] However, prolonged or repetitive HBO_2 therapy may cause excessive generation of ROS in rat lung[5] and in patients.[6] Neuroprotection by HBO_2 after MCAO is not found to be associated with decreased lipid peroxidation.[7] It remains unclear whether repetitive HBO_2 therapy provides better effects on brain inflammation and oxidative damage in rats with focal cerebral ischemia.

This study was conducted in order to evaluate the efficacy of a 1-day trial (2 HBO_2 treatments in one day) or 2—4-day trial (2 HBO_2 treatments daily and consecutively for 2—4 days) of HBO_2 therapy on both brain inflammation and oxidative stress caused by transient focal cerebral ischemia in rats. Therefore, by using the transient MCAO rat model, brain levels of proinflammatory cytokines [interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α)], an anti-inflammatory cytokine (IL-10), and a leukocyte accumulation indicator [myeloperoxidase (MPO) activity] were measured as indicators for brain inflammation. By contrast, brain levels of malondialdehyde (MDA), glutathione peroxidase (GPx), glutathione reductase (GR), superoxide dismutase (SOD), catalase, nitric oxide (NO), and 2,3-dihydroxybenzoic acid (2,3-DHBA) were measured as indicators for oxidative stress. The expected results would elucidate whether heightened oxygen tension by repetitive HBO_2 reduced oxidative stress, which was reflected by increased antioxidant and decreased oxidant contents under focal cerebral ischemia.

Methods

Animals

Adult male Sprague—Dawley rats (Animal Research Center of the National Science of the Republic of China (Taipei, Taiwan)) (weight, 246 ± 8 g) were housed under environmental conditions with ambient temperature of 22 ± 1°C, relative humidity of 65% and 12-hour light/dark cycle, with free access to food and water. Brain focal ischemia was induced by MCAO in rats by intraluminal filament, using the relatively noninvasive technique previously described by Belayev et al.[8] To allow reperfusion, the nylon filament was withdrawn 90 minutes after MCAO. The anesthetized animals were allowed to awaken and were kept in their cages with free access to food and water. All protocols, designed to minimize discomfort in the animals during surgery and in the recovery period, were approved by the Institutional Animal Care and Use Committee of Chi Mei Medical Center (Tainan, Taiwan) with a reference number of IACUC Approval No: 100120717.

HBO_2 therapy and animal groups

There were 192 rats randomly assigned to one of six groups: MCAO0T (MCAO rats untreated and euthanized 7 days post-MCAO; $n = 32$); MCAO1T (MCAO rats treated 3 hours after surgery with HBO_2 twice for 1 day and euthanized 7 days post-MCAO; $n = 32$); MCAO2T (MCAO rats treated 3 hours after surgery with HBO_2 twice daily for consecutive 2 days and euthanized 7 days post-MCAO; $n = 32$); MCAO3T (MCAO rats treated 3 hours after surgery with HBO_2 twice a day for consecutive 3 days and killed 7 days post-MCAO; $n = 32$); MCAO4T (MCAO rats treated 3 hours after surgery with HBO_2 twice a day for consecutive 4 days and euthanized 7 days post-MCAO; $n = 32$); and sham (sham-MCAO rats untreated and euthanized 7 days post-MCAO; $n = 32$). All tests were done with researchers blinded to which groups the rats were in; group codes were revealed only after all behavioral and histologic analyses had been completed. In MCAO groups treated with HBO_2, rats were placed in a custom-made pressure chamber of transparent acrylic plastic (Space Research Institute, Beijing, China) and given 1 hour of HBO_2 at 2.0 ata in 100% O_2 twice a day for 1—4 days. The chamber was flushed with 100% oxygen at a rate of 5 L/minute to avoid carbon dioxide accumulation. Decompression was done at 0.2 kg/cm^2/minute. During HBO_2 exposure, oxygen and carbon dioxide content were continuously monitored and maintained at ≥98% O_2 and ≤0.03% CO_2. The pressure chamber temperature was maintained between 22°C and 25°C. To minimize the effects of diurnal variation, all HBO_2 exposures were started at approximately 2:00 PM. MCAO-0 T or sham-MCAO was treated with normobaric air at 1.0 ata in 21% oxygen at an ambient temperature of 22—25°C.

Neurological and motor function evaluation

All rats were evaluated using a neurological severity score (NSS),[9] which is a composite of the motor (muscle status, abnormal movement), sensory (visual, tactile, and proprioceptive) and reflex tests. The inclined plane was used to measure limb strength. Animals were placed, facing right and left, perpendicular to the slope of a 20 cm × 20 cm ruffer ribbed surface of an inclined plane starting at angle